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INCREASED UPTAKE OF FATTY ACIDS BY THE ISOLATED RAT LIVER AFTER RAISING THE FATTY ACID BINDING PROTEIN CONCENTRATION WITH CLOFIBRATE

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Summary: Following the administration of clofibrate to rats, the concentration of Z protein or fatty acid binding protein in liver cytosol increases by 98 %. Ligandin concentration remains unchanged. Isolated perfused livers of clofibrate-treated rats take up free fatty acids from the perfusate at a significantly higher rate (+ 76 %) than controls. Lipid synthesis from radioactive fatty acids is not modified by clofibrate administration. The yield of plasma membranes obtained from liver homogenates as well as their lipid composition are similar in control and clofibrate treated livers. These results seem to exclude the possibility that the enhancement of FFA uptake could result from an indirect effect of the drug on FFA metabolism and/or plasma membrane surface and thus support the view that Z protein plays a role in intracellular fatty acid transport in the liver.

Introduction: Free fatty acids (FFA) are highly insoluble in water and must thus be transported in blood plasma bound to albumin and in biological media in the form of water soluble compounds (acyl-CoA or acyl-carnitine). Besides these well known acyl derivatives, Ockner suggested that a cytosolic protein (M.W. 12,000 daltons), fatty acid binding protein (FABP) which can be isolated from the intestinal mucosa, could play a role in the absorption, intracellular transport and esterification of fatty acids (1-3). In liver, Z protein, which is probably identical to FABP could also be involved in fatty acid transport and metabolism (4-6). Although contradictory results have been published (7-8)

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ABBREVIATIONS: FFA: free fatty acids; FABP: fatty acid binding protein; CLF: clofibrate; b.w.: body weight; M.W.: molecular weight; BSP: bromosulfophthalein.

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recent reports suggested the participation of Z protein in fatty acid uptake by normal isolated hepatocytes (9-10). Presumably, the reduced concentration of Z protein in Morris hepatoma cells (11) might be responsible for their abnormal lipid metabolism.

The fact that a variety of cholephilic anions carried by Z protein in the liver cell depressed the rate of uptake of FFA by perfused rat liver (12) probably reflects a competition for Z protein binding. This competition has been already demonstrated in liver cytosol fractions in vitro (4). We postulate in this study that if the Z protein plays a role in FFA uptake, a modification of its concentration in hepatic cytosol should influence the FFA uptake rate by the isolated liver.

MATERIALS AND METHODS: Male Sprague Dawley rats weighing 200 g b.w. were given saline (control rats) or clofibrate (200 $mg.kg^{-1}$) (clofibrate treated rats) via a gastric tube every 12 hours for 4 days and were fed ad libitum until sacrifice. Liver perfusions were performed as described elsewhere (13). Briefly, a standard perfusate composed of rat blood diluted with Krebs-Ringer buffer (1:2, v/v) containing 4 g/100 ml of albumin was first recirculated for 20 minutes. During the equilibration period, transhepatic blood flow, bile secretion rate and the pH, pO_2 and pCO_2 of the perfusate were monitored and maintained at physiological values. At the end of the equilibration period the portal cannula was connected to a reservoir containing fresh Krebs-Ringer buffer with [^{14}C]palmitate (specific radioactivity: 49.2 mCi/nmole) bound to albumin. The FFA/albumin molar ratio was 0.7. The effluent perfusate was collected every 30 seconds for 10 minutes. The transhepatic flow rate was maintained constant at 1 $ml.min^{-1}.g\ liver^{-1}$ by adjusting the hydrostatic pressure. The difference between plasma FFA concentration (14) and radioactivity in the portal and in the hepatic veins, gives the FFA uptake rate during a single pass of the perfusate through the liver (15). At the end of the perfusion experiments, livers were weighed and perfused through the hepatic veins with 50 ml of ice-cold isotonic saline. Total lipids from 1 g of liver were extracted (16) and fractionated by thin layer chromatography in order to study the utilization of fatty acids taken up by the liver. The preparation of Y and Z fractions from the cytosol of liver and the determination of their BSP binding capacity were described previously (17). In other experiments, plasma membranes (18) and microsomal fractions (19) were isolated from rat liver homogenates. Their protein (20) phospholipid (21) and cholesterol (22) contents were determined.

STATISTICAL METHODS: The significance of differences in FFA uptake between control and clofibrate-treated rats was determined with the Student's t test.

TABLE 1 CYTOSOL CONTENT AND BSP BINDING CAPACITY OF Y AND Z PROTEINS IN RAT LIVER

	Y		Z	
	PROTEIN	BINDING CAPACITY	PROTEIN	BINDING CAPACITY
	mg.g liver^{-1}	nmoles BSP.g liver^{-1}	mg.g liver^{-1}	nmoles BSP.g liver^{-1}
CONTROL RATS	6.15 ± 0.24	103 ± 4	1.93 ± 0.09	63 ± 1
CLOFIBRATE-TREATED RATS	6.43 ± 0.24	94 ± 5	3.83 ± 0.36	163 ± 19
STUDENT'S T TEST	n.s.	n.s.	$p < 0.001$	$p < 0.001$

All data are given as the Mean S.E.M. from 6 rats per group (n.s.=not significant)
Y and Z fractions were prepared from rat liver homogenates as described in MATERIALS and METHODS.

RESULTS AND DISCUSSION: In the cytosol following clofibrate administration, ligandin (Y protein) concentration and its BSP binding capacity remain unchanged. On the contrary, the concentration of Z protein sharply increases by 98 % and its BSP binding capacity increases by 160 % (Table 1) compared to control values, agreeing with previously published studies (23). Isolated livers from clofibrate-treated rats take up 75 % more fatty acid than those from control rats. The percentage of perfusate FFA taken up during a single pass through the liver is increased by 66 %. Consequently, the hepatic uptake capacity for FFA is increased by clofibrate (Table 2). It has been reported that addition of clofibrate to a suspension of Ehrlich ascites cells increases the FFA uptake rate; however, this phenomenon results from a displacement of fatty acid from albumin binding sites (24). In our experiments, such an effect of clofibrate cannot be involved since there is no drug in the perfusion medium and blood is flushed from livers by isotonic saline prior to the experiments.

TABLE 2 UPTAKE RATE OF F.F.A. BY THE PERFUSED RAT LIVER

	$nEq.g^{-1}.min^{-1}$	% of uptake
CONTROL RATS	92.9 ± 7.3	19.7 ± 1.1
CLOFIBRATE-TREATED RATS	162.2 ± 12.9	32.7 ± 3.2
STUDENT'S T TEST	$p < 0.01$	$p < 0.01$

The results represent the mean ± S.E.M. from 6 rats per group.

The uptake rate was determined as described in MATERIAL AND METHODS

Fatty acids taken up by the liver are rapidly incorporated into phospholipids and triglycerides. A minor proportion of radioactivity (< 5%) was found as FFA in the hepatic lipid extract. The incorporation of [^{14}C] palmitate into phospholipids and triglycerides (Table 3) is similar in control and clofibrate rats when the results are expressed per g of liver and is not significantly increased in CLF animals when the results are expressed on a total liver basis, since clofibrate treatment increases liver weight by 25 % (control rats: 3.7 g/100 g b.w.; clofibrate-treated rats: 4.7. g/100 g b.w.; $p < 0.001$).

The lipid composition of total rat liver is not significantly modified by clofibrate (Table 3) in accordance with previously published results (25). The recovery of radioactive fatty acid as hepatic lipid is not significantly increased by CLF administration in spite of the

TABLE 3

COMPOSITION AND RADIOACTIVITY OF LIVER LIPIDS

	PHOSPHOLIPIDS			TRIGLYCERIDES			CHOLESTEROL
	mg.g liver^{-1}	cpm.g^{-1}.10^{-3}	cpm.10^{-3}.liver^{-1}	mg.g liver^{-1}	cpm.g^{-1}.10^{-3}	cpm.10^{-3}.liver^{-1}	mg.g liver^{-1}
CONTROL RATS	36.0 ± 2.8	36.4 ± 5.3	269.7 ± 18.8	6.0 ± 0.4	44.8 ± 8.9	347.5 ± 80.0	2.3 ± 0.2
CLOFIBRATE-TREATED RATS	39.3 ± 1.5	31.5 ± 3.6	322.0 ± 47.3	5.9 ± 1.2	46.3 ± 6.7	472.4 ± 69.3	1.9 ± 0.2
STUDENT'S T TEST	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

All data are given as the mean ± S.E.M. from 6 rats per group and determined as described in MATERIALS AND METHODS

n.s. = not significant

TABLE 4 PROTEIN CONTENT AND LIPID COMPOSITION OF RAT LIVER PLASMA MEMBRANES AND MICROSOMES

	PLASMA MEMBRANES				MICROSOMES		
	PROTEIN mg.g liver^{-1}	CHOLESTEROL (CS) µmoles.mg prot.$^{-1}$	PHOSPHOLIPIDS (PL) µmoles.mg prot.$^{-1}$	CS/PL molar ratio	CHOLESTEROL (CS) nmoles.g prot.$^{-1}$	PHOSPHOLIPIDS (PL) nmoles.mg prot.$^{-1}$	CS/PL molar ratio
CONTROL RATS	0.93 ± 0.10	0.34 ± 0.01	1.01 ± 0.12	0.35 ± 0.04	63 ± 6	520 ± 40	0.122 ± 0.003
CLOFIBRATE-TREATED RATS	1.10 ± 0.01	0.32 ± 0.01	0.81 ± 0.01	0.40 ± 0.02	52 ± 3	620 ± 30	0.081 ± 0.003
STUDENT'S T TEST	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	$p < 0.001$

All data are given as the mean ± S.E.M. from 6 rats per group.

n.s. = not significant

fact that these livers take up 75 % more fatty acids than the controls. Although fatty acid catabolism was not directly measured in our experiments, it may be assumed that the "excess" of fatty acid taken up by CLF treated livers is primarily directed to oxidation pathways in mitochondria and peroxisomes. The fatty acyl CoA oxidizing capacity in these organelles has been reported to be enhanced by clofibrate (26-28).

Clofibrate administration does not increase the recovery of plasma membranes. In addition, their protein content and lipid composition remain unchanged (Table 4), suggesting that the surface of the sinusoidal plasma membrane is not affected by the drug, which is in agreement with electron microscopic observations (26).

Clofibrate administration decreases the cholesterol/phospholipid molar ratio in the purified microsomal membranes (Table 4). This decrease is also observed after treatment with phenobarbital and other "microsomal enzyme inducers" which produce a hyperplasia of the smooth endoplasmic reticulum (29).

In conclusion, clofibrate administration to rats induces changes in hepatic metabolism and ultrastructure, including a significant increase of fatty acid uptake and a sharp rise of the FABP or Z protein content of the liver cell. Both findings can be correlated with other published findings which suggest that Z protein acts as a fatty acid carrier in the cytosol. Although other interpretations cannot be defitively excluded, two major alternatives (increased plasma membrane surface and/or increased fatty acid esterification) that could theoretically influence the rate of fatty acid uptake are not supported by our results. This contributes new experimental arguments to the hypothesis that FABP is involved in the uptake and perhaps the metabolism of fatty acids in hepatocytes.

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